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TITLE: Central Pain Mechanisms and Novel Therapeutic Strategies in a Model of Closed Head Injury

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14. ABSTRACT Headache is the most common, persistent symptom of post-concussion syndrome and highly prevalent following traumatic brain injury of all severities. Inflammation is an early promoter of pain, and is proposed to play an important role in the pathogenesis of chronic post-traumatic headache; however, this role is not well defined. This research investigates the contribution of acute and chronic inflammation to the development of headache after closed head injury. The specific aim (1) was to determine the pattern of inflammation-induced sensitization of the central trigeminal pain neurons, and if sensitization is detectable by quantitative EEG. Sprague Dawley rats underwent mild closed head injury (CHI) or served as an incision control group to determine the effects of graded inflammatory on central trigeminal pain neurons. CHI groups were subdivided into single CHI _{x1} or repeated two (CHI _{x2}) and three (CHI _{x3}) injury groups to determine the graded effects of inflammation. Acute endpoints were at 1 day and 1 week, and the chronic endpoints were at 4 weeks. Quantitative electroencephalography shows group differences in several frequency and cortices that persist over time and respond to light stimuli. Headache behavioral testing as well as immunohistochemical and molecular studies were used to uncover the underlying inflammatory contributions to post-traumatic headache. Outcomes from injury are dependent on the number of injuries and injury force parameters.					
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- **INTRODUCTION:**

Headache is the most common, persistent symptom of post-concussion syndrome and highly prevalent following traumatic brain injury of all severities. Inflammation is an early promoter of pain, and is proposed to play an important role in the pathogenesis of chronic post-traumatic headache; however, this role is not well defined. This research investigates the contribution of acute and chronic inflammation to the development of headache after closed head injury. The specific aim (1) was to determine the pattern of inflammation-induced sensitization of the central trigeminal pain neurons, and if sensitization is detectable by quantitative EEG. Sprague Dawley rats underwent mild closed head injury (CHI) or served as an incision control group to determine the effects of graded inflammatory on central trigeminal pain neurons. CHI groups were subdivided into single CHI_{x1} or repeated two (CHI_{x2}) and three (CHI_{x3}) injury groups to determine the graded effects of inflammation. Acute endpoints were at 1 day and 1 week, and the chronic endpoints were at 4 weeks. Quantitative electroencephalography, headache behavioral testing, as well as immunohistochemical and molecular studies were used to uncover the underlying inflammatory contributions to post-traumatic headache..

- **KEYWORDS:**

Post-traumatic headache

Post-traumatic migraine

Chronic migraine

Traumatic brain injury

Quantitative EEG (QEEG)

Analgesia

Endocannabinoid

Cannabinoid receptors

Cannabinoid type 2 receptor

- **ACCOMPLISHMENTS:**
- **What were the major goals of the project?**

Specific Aim 1 was to determine the pattern of inflammation-induced sensitization of the central trigeminal pain neurons, and if sensitization is detectable by quantitative EEG.

Table 1: Estimated completion dates for Year 1		
Major Task	Projected completion Date	% Completed
1: Conduct quantitative EEG testing in a pre-clinical model of concussion	3/30/15	75%
2: Conduct behavioral testing in rats	12/30/15	100%
3: Completion of post-mortem histology and molecular studies for 3 study endpoints	12/30/15	95%

- **What was accomplished under these goals?**

1) **Major Activities** for year 1, specific aim 1:

- Generated acute and chronic cohorts for mild closed head injury and incision control groups as described under specific aim 1.
- Completed 7 experimental cohorts of chronic (4 week) closed head injury groups for EEG studies. On our current EEG system, *only 4 rats can be run at a time for a 4-week period.*
- Completed installation, calibration and testing of a new EEG system from Pinnacle. Hired and trained an upper level Research Associate (Dr. Lan Cheng, MD, PhD) to perform EEG studies at Thomas Jefferson University following the Co-I on this project, Dr. Chin, moving to another institution in June of 2015.
- Completed behavioral testing indicative of post-traumatic headache including allodynia testing and light aversion/light sensitivity.
- Conducted post-mortem immunohistochemical, western blot and qRT-PCR studies.

2) Study Objectives: The first objective was to determine the pattern of inflammation-induced sensitization of the central trigeminal pain neurons, and if sensitization is detectable by quantitative EEG.

Subaim 1a) Conduct quantitative EEG testing in a pre-clinical model of concussion

Subaim 1b) Conduct behavioral testing in rats

Subaim 1c) Completion of post-mortem histology and molecular studies for 3 study endpoints

3) Results and Conclusions:

Sprague Dawley rats underwent single mild closed head injury (sCHI), repeated (rCHI) or served as an incision control group to determine the effect of mild CHI on graded inflammatory induced sensitization of central trigeminal pain neurons. CHI groups were subdivided into single CHI1 or repeated two (CHI2) and three (CHI3) injury groups. Acute endpoints were at 1 day and 1 week, and the chronic endpoint will be at 4 weeks. We have completed generating groups for acute and chronic cohorts. Results for quantitative EEG, behavioral, immunohistochemical and molecular studies are described below.

EEG power analysis shows a graded effect as a function of the number of injuries and group differences in power output following CHI2 and CHI3 compared to incision control groups. Data for rCHI groups undergoing three (3) CHI3 showed a substantial difference in the EEG power analysis of the bilateral frontal and parietal cortex power for theta (4-10Hz) and beta (10-25Hz) and gamma (40-100Hz) frequencies compared to our previous CHI groups undergoing two (2) CHI2 (**Figure 1**). EEG power analysis of the frontal and parietal (left and right) cortical electrodes showed that for both the CHI3 animals (n=2) and the incision control (n=1), the power of the theta (4-10 Hz) and beta (10-25 Hz) frequency bands was greater than for the gamma frequency band (40-100 Hz). Furthermore, EEG powers over the two parietal cortices were markedly larger than for the frontal cortex. Notably, the power analysis also revealed an overall increase in power in CHI3 rats compared to the incision control animal. This increase in power was particularly pronounced for the theta and beta frequency bands in every cortical location, while for the gamma frequency band the increase was relatively modest and seen primarily over the two parietal cortices. **Theta and beta frequency power in the frontal cortex was higher in CHI3 animals from the initial week of recordings, and remained increased throughout the 4 weeks. These results demonstrate that CHI3 animals display EEG biomarkers that develop and are stable over time, for at least the one month duration of monitoring.**

EEG power analysis of the bilateral frontal and parietal cortex reveal increased power for theta (4-10Hz) and beta (10-25Hz), but not gamma (40-100Hz) frequencies as well in rCHI2 rats compared to the control. Specifically, substantial changes continue to be noted on the power analysis of the frontal cortex theta frequencies (**Figure 2A**) and parietal cortex EEG which reveals overall higher power for theta and beta frequencies in rCHI animals compared to the control prior to photostimulation (**Figure 2 D, E**). All rCHI2 rats consistently showed higher power for the theta frequencies on the parietal cortex EEG compared to the control rat.

Photostimulation did not alter the power of any frequencies in the frontal or parietal cortices in any of the controls, but produced an increase in the overall theta frequencies of rCHIx2 animals. Although the overall pre-photostimulation power analysis was higher for all individual rCHI rats compared to controls, only 40% of rCHI rats responded to photostimulation. These rates are similar to those reported in humans with post-traumatic headache by Hoffman et al.¹ This variability is expected and was noted in our last report. The data presented in figure 1 shows the 3 out of 5 responders to photostimulation.

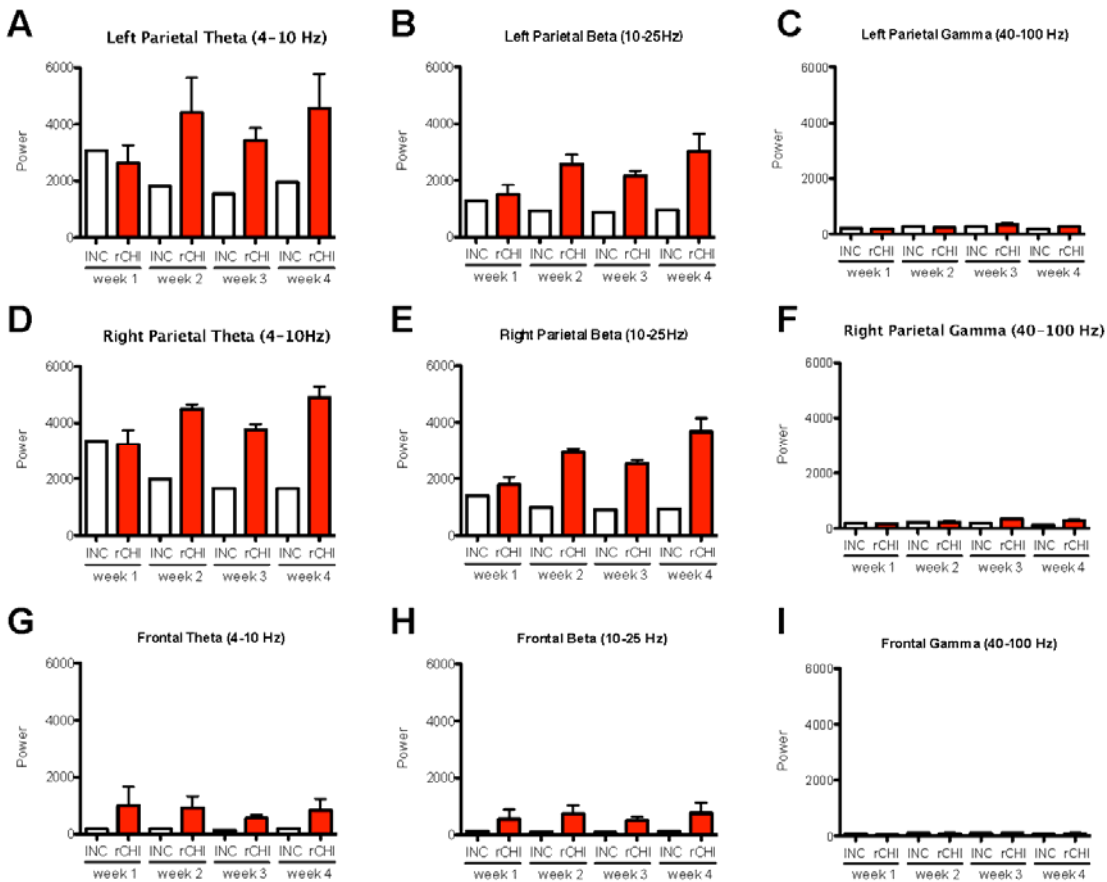


Figure 1: Quantitative power analysis of EEG waveforms. QEEG from rats with repeated closed-head injuries (rCHI, n=2) and an incision control rat (INC), across 4 weeks of recordings (one 24-hour recording per week, for four weeks). Data are displayed from 3 recording locations along the animals' skulls: left (A-C) and right (D-F) parietal cortices and bilateral frontal cortex (G-I). Total power values for each cortical location are displayed for theta (4-10Hz, A, D, G), beta (10-25Hz, B, E, H), and gamma (40-100Hz, C, F, I) frequencies. Error bars represent SEM.

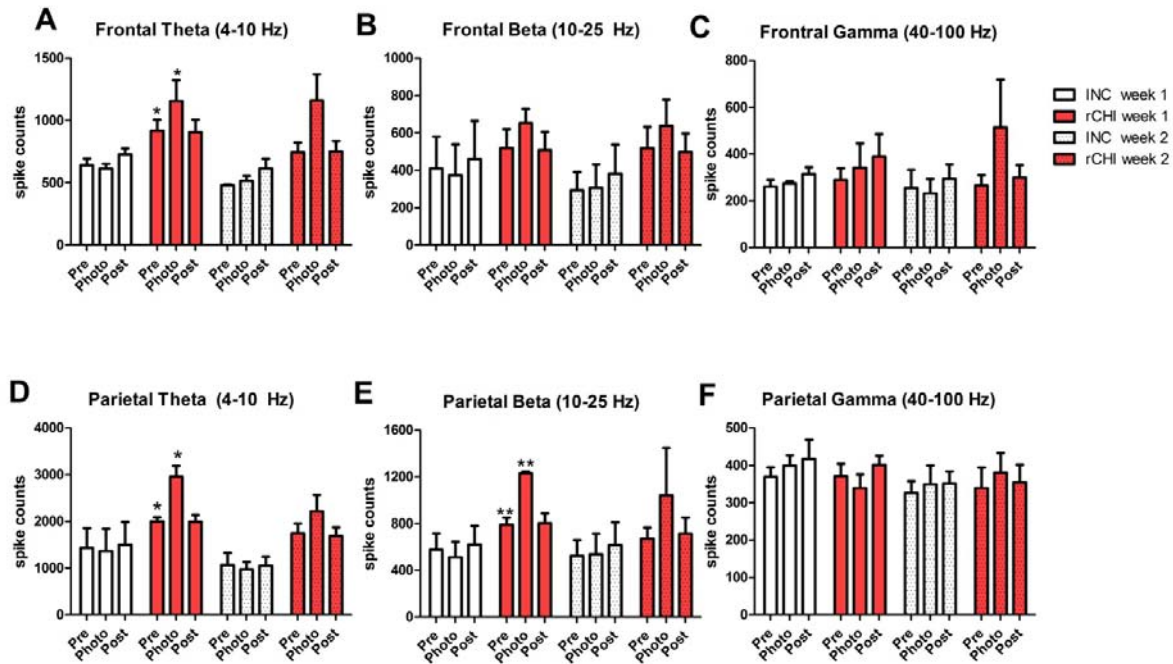


Figure 2: Quantitative power analysis from EEG recordings of the frontal and parietal cortices of animals with repeated closed-head injuries (*rCHI*, $n=3$) and an (*incision*, $n=3$) control rats, pre-stimulation (pre), during photostimulation (photo) and post-photostimulation (post). Note: Two *rCHI* rats with higher pre-stimulation power analysis for frontal and parietal theta and beta frequencies were non-responders to photostimulation (not shown). Power analysis of **frontal cortex (A-C)** and **parietal cortex (D-F)** EEG for theta frequencies (4-10Hz), beta (10-25Hz), and gamma (40-100Hz) frequencies, in *rCHI* rats (red bars) compared the control rat (white bars) during baseline (pre) monitoring, photostimulation (photo), and post-monitoring (post) periods at week 1 (solid bars) and week 2 (dotted bars) after injury. (A) * $p<0.05$ compared to Incision pre and *rCHI* pre; (D) * $p<0.05$ compared to Incision pre and *rCHI* pre; (E) ** $p<0.01$ compared to Incision pre and *rCHI* pre.

Analysis of the chronic marker of neuronal activation, deltaFosB, show an increase in the left and right sensory cortex after repeated CHI compared to incision controls. (**Figure 3**). This provides evidence of enhanced chronic neuronal activity in the injured group in the sensory cortex. The thalamus regions did not show changes in deltaFosB in any groups (not shown); however, this may be due to the large thalamic area analyzed and a dilutional effect. It is possible that subnuclei within the thalamus show changes that could not be found in these samples.

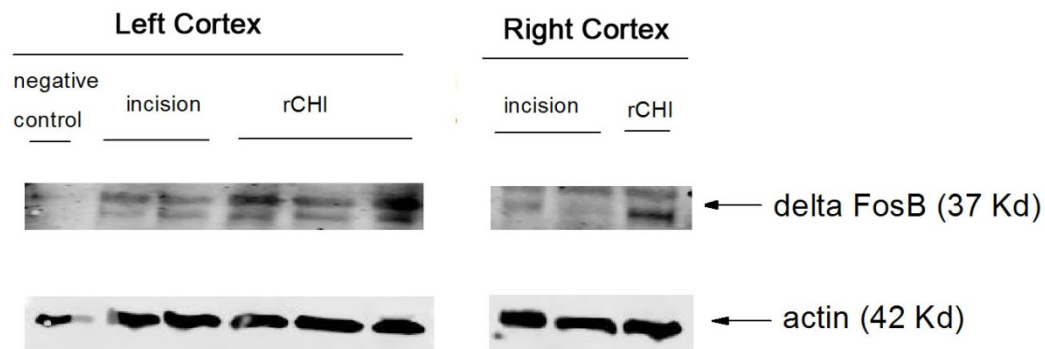


Figure 3: Preliminary Western Blot showing Delta Fos B in sensory cortex of incision and rCHI rats. Delta Fos B (37 Kd) and actin (42 Kd). Negative wild-type murine control tissue show negligible delta Fos B.

Inflammation initiates pain signaling after head trauma in which microglia phenotype changes in the TNC after rCHIx2 at one day after injury were reported previously. Microglia play an important role in other neuropathic pain models and show altered phenotypes in the spinal cord after insults to peripheral nerves. We have found phenotype changes in the thalamus (**Figure 4**), as TNC (**Figure 5**) that are undergoing a more in depth investigation. Microglial are the predominant cell source for pro-inflammatory mediators including cytokines (TNF- α) and inducible nitric oxide synthase (iNOS) released near injured tissues. Increased iNOS is found in the thalamus after repeated CHI (CHIx2) in two specific nuclei, the reticular thalamic nuclei and posterior thalamic nuclei but was absent in the VPM/VPL pain region. These other thalamic regions are involved in pain as well and further investigations continue to study this pathway. In addition, we determined the cell source for the iNOS product to be neuronal and not microglial in the thalamus (**Figure 6B**). Together, the positive finding for enhance neuronal activity in the sensory cortex and NOS signaling in the thalamus warrant further investigation.

Interestingly, these microglial changes are in the absence of axonal injury as assessed using beta-amyloid precursor protein (APP) staining. Axonal injury is evidenced by an accumulation of beta amyloid precursor protein in the axon and soma of the neuron. Evidence of axonal injury in other thalamic zones was also noted that is dependent on the number of injury (**Figure 7-8**). An important point to note it that the axonal injury is absent from the VPM/VPL area of the thalamus involved in relaying pain; rather the axonal injury is found in the most ventral aspect of the posterior nuclei of the thalamus. Inflammatory proteins including the cytokine TNF-alpha and prostaglandin E2 did not show robust changes in the forebrain tissue or in the microdissected thalamus (**Figure 9-10**). This negative finding is most likely due to the dissection technique and a dilutional effect on the samples. We plan to do an even finer microdissection of the thalamic subnuclei (Po, VpM, VpL, nRT) in a small group of animals for our next assays.

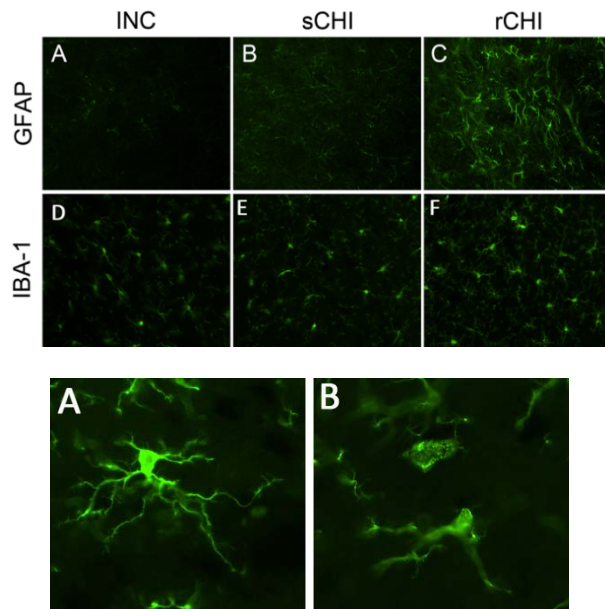


Figure 4: TOP: Qualitative immunohistochemical analyses of astrogliosis (GFAP) and microgliosis (IBA-1) in a thalamus pain region for incision control (A,D), sCHI (B,E), and rCHI (C,F).

BOTTOM: Images showing morphological analysis of phenotype switch in IBA-1 positive microglia for incision (A) and rCHI (B) in thalamic pain region. (A) Resting microglia showing a highly ramified morphology. (B) Activated microglia showing retracted processes, enlarged soma, and amoeboid morphology.

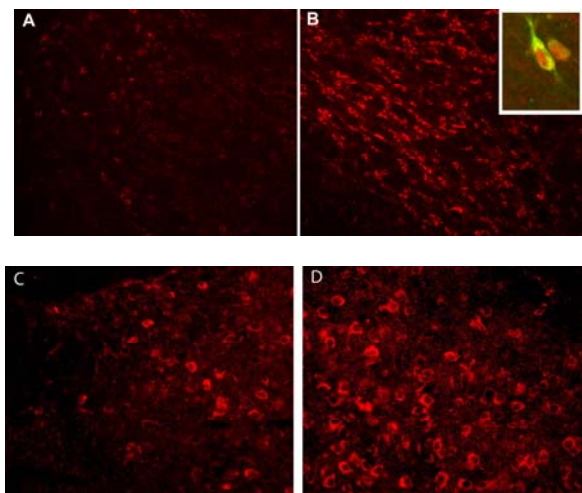


Figure 6. Thalamic nuclei s of control rat (A,C) and rCHI (B,D). Inset shows neuronal nuclear stain (red) is colocalized with iNOS (yellow-green). Incision rat for reticular thalamic nucleus (nRT) (A) and posterior nucleus (Po) show minimal iNOS labeling. CHIx2 shows increased iNOS immunoreactive neurons in the nRT (B) and Po (D).

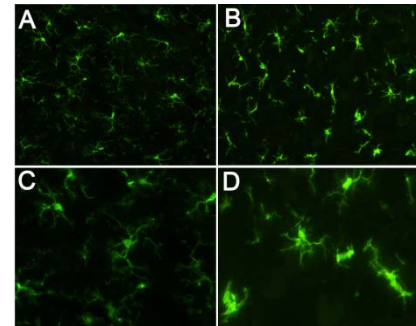


Figure 5. Ionized calcium binding adaptor molecule 1 (Iba1) labeled microglia in the trigeminal nucleus caudalis of incision control (A, C) and repeated closed head injured (B, D) rats. Noted resting, ramified morphology in controls (A,D) and altered microglial phenotype in rCHI rat TNC (B, D).

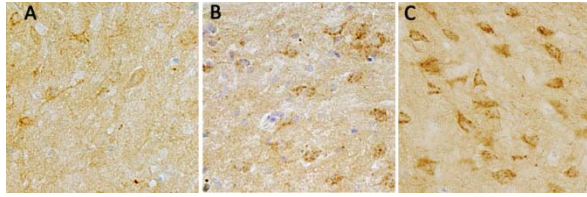


Figure 7: β -Amyloid Precursor Protein (β -APP) immunostaining for incision control (A), sCHI (B), and rCHI (B) in the lateral dorsal thalamic non-pain associated region.



Figure 8: β -APP immunostaining for incision control (A), sCHI (B), and rCHI (C) in the TNC pain region.

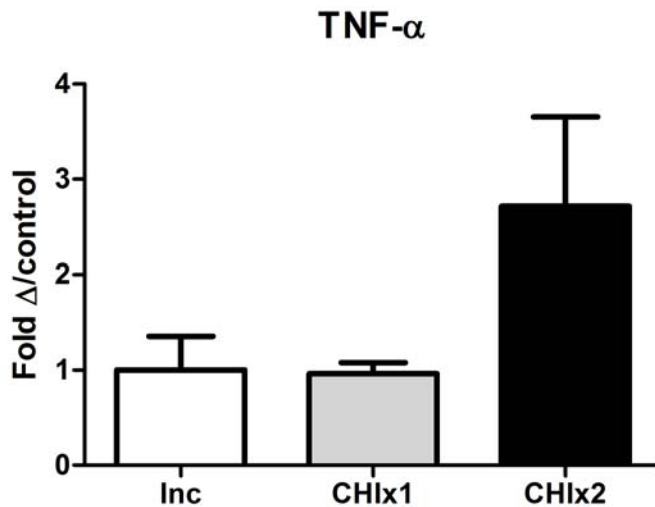


Figure 9. TNF-alpha protein levels measured using ELISA for incision control (n=6), single CHI1 (n=2), and repeated CHI2 (n=10) groups (ANOVA not significant p=0.27).

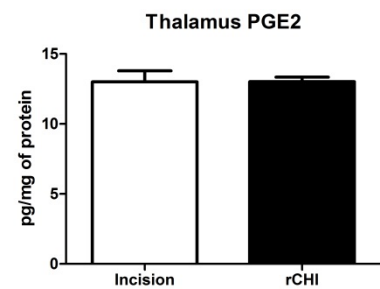


Figure 10. Prostaglandin E2 (PGE2) levels in the thalamus in rCHI (n=4) and incision controls (n=5).

Previously we showed increases in calcitonin gene-related peptide (CGRP) in the TNC at the 1 day endpoint that persist at the 4 week end point. CGRP levels in the TNC after injury were significantly increased in rCHI2 rats compared to incision but not after a single CHI, $p < 0.001$ (**Figure 11**). CGRP immunoreactivity is negligible in incision controls, whereas there is robust CGRP immunoreactivity shown at 1 day and up to 4 weeks in rCHI2 rat TNC (**Figure 12**). CGRP immunoreactivity is dependent on the number of injuries as shown for 1 day groups (**Figure 13**). Increases at one week after injury indicates persistent sensitization of the trigeminal ganglia neurons and meningeal nociceptors. iNOS appears in a similar distribution as CGRP in the TNC (**Figure 14**) most likely

released from the afferent terminals of the trigeminal ganglia. We propose this is the input or driver to the thalamus.

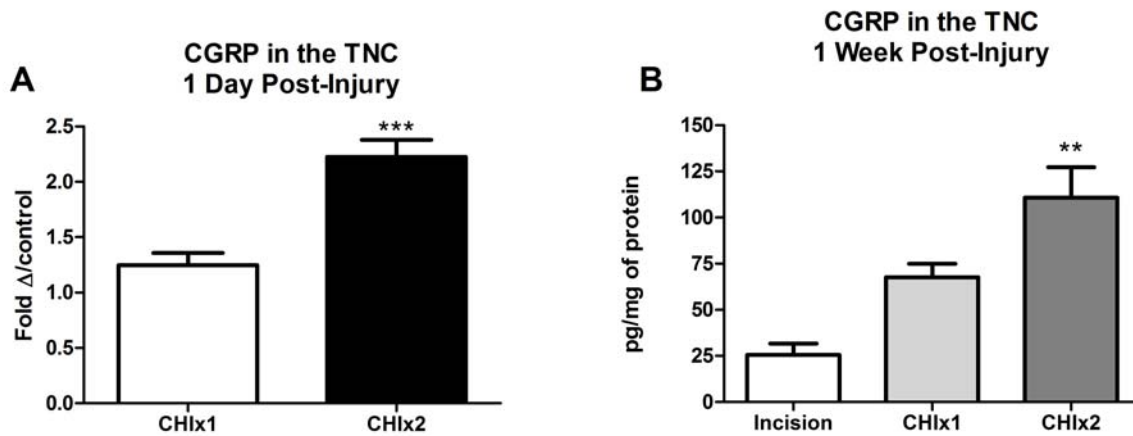


Figure 11: CGRP enzyme linked immunosorbant assay used to assess the trigeminal nucleus caudalis (TNC) at 1 day (A) and 1 week (B) post injury. Single CHI (CHIx2) and repeated CHI (CHIx2) and Incision groups were compared after injury, *** $p < 0.001$ (A) and ** $p < 0.01$ (B). A) CHIx1 $n=6$, CHIx2 $n=6$ B) Incision $n=4$, CHIx1 $n=5$, CHIx2 $n=6$

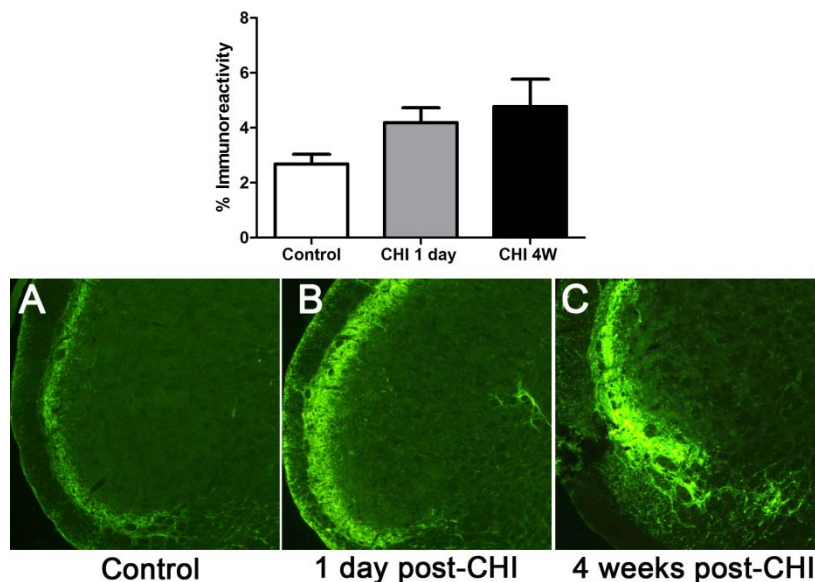


Figure 12: Anti-calcitonin gene-related peptide (CGRP) immunohistochemical analysis shows robust immunoreactivity at 1 day and 4 weeks after injury compared to incision controls. Controls $n=2$, CHI 1 day and 4 weeks $n=4$ /group.

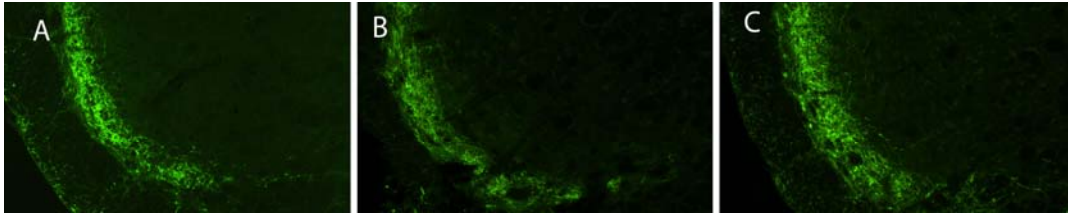


Figure 13: Calcitonin gene-related peptide (CGRP) robust immunoreactivity shown for incision controls (A) and single closed head injured (B), and CHI_x2 rats (C) at 1 day post-operatively. There is a wider spread of CGRP noted in the CHI animals receiving two injuries (C) compared to no injury (A) or a single injury (B).

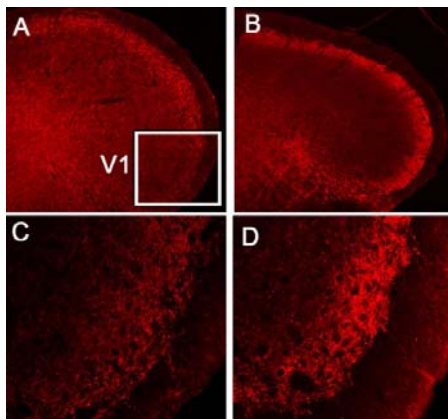


Figure 14: iNOS immunoreactivity (iNOR-IR) in the trigeminal nucleus caudalis. iNOS-IR in single CHI (A) and repeated CHI (B) is shown. There is a visible increase in iNOS immunoreactivity in rCHI (B, D) compared to sCHI (A,C).

Trigeminal allodynia were testing using von Frey sensory testing over four weeks after injury. Normal thresholds are 8 to 10 grams, whereas 6 grams or less is considered allodynia (Figure 15; dotted line indicates allodynia). We found a significant reduction in sensory thresholds (allodynia) in all CHI groups (**Figure 15**). Allodynia in CHI rats with two and three injuries persisted for four weeks while single CHI (CHI_x1) returned to baseline levels by 4 weeks. Findings indicated a graded response that is dependent on the number of head injuries as well as force of injury. Force of injury was dependent on the displacement (2 mm or 5 mm) while the velocity of impact was held constant (5 m/s). At one week post-injury, 67% of CHI_x1 rats showed allodynia, while 100% of CHI_x2 rats and CHI_x3 rats showed allodynia. By 4 weeks, there were no CHI_x1 rat showing allodynia, while 33% of CHI_x3 continues to show allodynia (4 out of 12); These rates are similar in some studies reporting 30-40% of PTH in humans. Stratifying the allodynic and non-allodynic chronic subgroups is warranted.

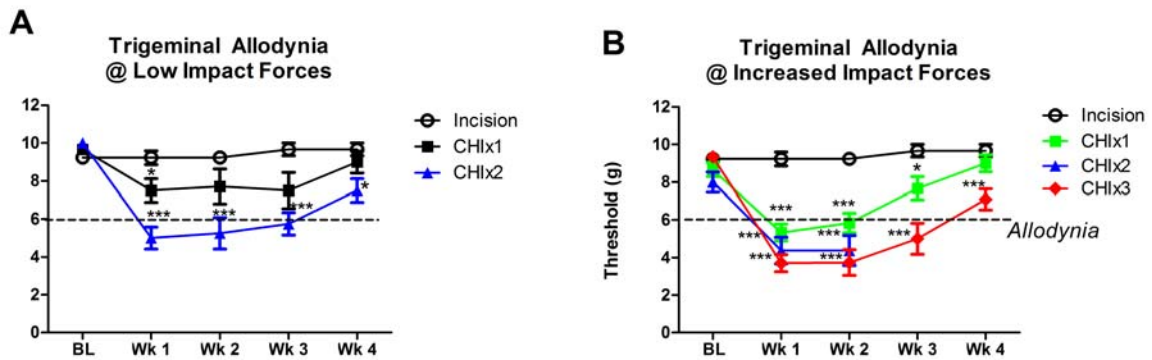


Figure 15: Trigeminal allodynia determined using von Frey sensory testing is a function of force and number of injury. Force is determined in part by acceleration in which the displacement of was either 2 mm or 3mm (A), or 5 mm (B) to create low or increased forces respectively. Trigeminal thresholds (grams) are shown for incision and mild closed head injured (CHI) rats with 1, 2 or 3 repeated injuries at baseline (BL) and weeks 1-4, * $p < 0.05$ and *** $p < 0.001$ compared to incision control. Allodynia persists for 4 weeks in animals with 3 hits under the increased forces compared to animals with a single hit and controls. A) 2 mm displacement Incision $n=9$, CHIx1 $n=1$, CHIx2 $n=8$ B) Incision $n=13$, CHIx1 $n=12$, CHIx2 $n=8$, CHIx3 $n=12$

Unmet goals due to Dr. Chin moving to another institution are on track to be completed in the next few months. In particular we have remaining qEEG studies ($n=12$ rats) to complete and (post-mortem western blot and qRT-PCR) to complete. Western blot data and qRT-PCR is expected to be completed in the next quarter as well. Tissues have been generated by Dr. Elliott's laboratory and the WB shown here was performed by Dr. Cheng in Dr. Elliott lab for deltaFosB in the interim while Dr. Chin set up her laboratory at Baylor. Future analyses will be conducted to determine potential factors contributing to the activation in this region by Dr. Chin's Laboratory.

Although we see evidence of photophobia behaviors, we have found a high variability in our light testing, which we do not find for our mouse models. To resolve this we have modified our protocols to include additional acclimation trials which we expect will resolve this issue. Because we are working with chronic cohorts, the trials take time to administer. If the problem continues in the next round of animals, there could also be an issue with the animal tracking due to the camera and color in which case we will then have our engineers construct a low cost insert for a dark background to improve the visualization of the white Sprague Dawley rats. We expect these issues to be resolved and to have the assay working in time for drug testing.

- **What opportunities for training and professional development has the project provided?** *Nothing to Report*

- **How were the results disseminated to communities of interest?** *Nothing to Report*

- **What do you plan to do during the next reporting period to accomplish the goals?**

- Complete histological analysis of remaining chronic groups including immunohistochemical and molecular assessments.
- Complete first manuscript preparation for submit for publication.
- Continue to generate remaining groups for the EEG analysis.
- Continue light testing protocols
- Genes will be analyzed in chronic groups and followed up with protein analysis
- Slice experiments including drug testing under aim 2 has been initiated and will continue.

- **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**
- Our findings have enhanced our understanding of the mechanisms underlying post-traumatic headache. In addition, the use of non-invasive EEG combined with light stimuli in patients with post-traumatic migraine is novel. Once published, results have the potential to directly impact the clinic in this population.
- **What was the impact on other disciplines?**
- Nothing to Report
- **What was the impact on technology transfer?** *Nothing to Report*
- **What was the impact on society beyond science and technology?**

Nothing to Report

- **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**

Nothing to Report

- **Actual or anticipated problems or delays and actions or plans to resolve them**

The co-I, Dr. Jeannie Chin, moved to Baylor University as of June 1, however, she remained as co-I on the project. The only tasks that moved to Baylor were the data analysis for EEG and some histology (western and qRT-PCR for FOS markers). All animal work remains the same at Thomas Jefferson University. A revised SOW and rebudgeting documentation was submitted on August 27, 2015 via the Thomas Jefferson University research administration. The EEG equipment remains in house at Jefferson and all EEG and slice experiments will be run at Jefferson because these need to be done soon after injury, which is performed by my laboratory.

EEG equipment was relocated from Dr. Chin's old laboratory to Dr. Elliott's laboratory and all equipments was set up, calibrated and tested. Dr. Lan Cheng (MD, PhD) was hired to work on this project and will lead the EEG experiments. She has an extensive background in electrophysiology, animal surgeries, as well as skills in molecular biology. Dr. Cheng has successfully implanted rats with EEG, and has been monitoring new cohorts of implanted rats. The new data set in this report that is collected was collected by Dr. Cheng on our new Pinnacle EEG set up.

We scheduled to deliver chronic CHI samples for Western Blot analysis to Dr. Chin's laboratory at Baylor once her laboratory and personnel was set up in September. Dr. Cheng ran one batch of samples in the interim until Dr. Chin's lab was setup, which is included in this annual progress report. We also lost a large shipment (15 samples/rats) due to improper handling and dry ice evaporation, in which we have since remedied the problem and resent new samples to Baylor. A successful West Blot run of samples will be included in the next quarterly report due January 14, 2015.

We have found a high variability in our light testing, which we do not find for our mouse models; we are modifying our protocols to include additional acclimation trials which we expect will resolve this issue. We may also construct a new insert to improve the visualization of the light colored rats by the camera during testing.

- **Changes that had a significant impact on expenditures**

Nothing to report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report

- **Significant changes in use or care of human subjects:**

Nothing to Report

- **Significant changes in use or care of vertebrate animals:**

Nothing to Report

- **Significant changes in use of biohazards and/or select agents:**

Nothing to Report

- **PRODUCTS:**

- **Publications, conference papers, and presentations**

Journal publications.

- **Books or other non-periodical, one-time publications.**

- **Other publications, conference papers, and presentations.**

Invited Speaker Presentation: Title: The Role of the Cannabinoid Receptor Type-2 in Head Trauma: Studies on Inflammation and Pain Mid-Atlantic Pharmacology Society, Thursday, October 22, 2015, Cooper Medical School Rowan University, Camden, NJ

- **Website(s) or other Internet site(s)**

<http://www.jefferson.edu/university/jmc/departments/neurosurgery/faculty/elliott.html>

- **Technologies or techniques** *Nothing to Report*

- **Inventions, patent applications, and/or licenses** *Nothing to Report*

- **Other Products:**

Biospecimen collections were generated for a portion of the acute and chronic study groups for concussion model and model of post-traumatic headache.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	Melanie Elliott	Jeannie Chin	Brittany Daiutolo	Ashley Tyburski	Mark Pyfer	Lan Cheng
Project Role:	PI	Co-I	Research Technician	Research Technician	Research Technician	Research Associate
Researcher ID:	n/a	n/a	n/a	n/a	n/a	n/a
Month worked:	2.5	1.0	3.6	6.0	1.2	.25
Contribution:	Experimental design, data interpretation and presentation	EEG Setup, Experiments	Surgeries, behavior, animal care	Behavior, animal care, immunohistochemistry	EEG setup, surgery, experiments	EEG surgery, PCR

- **What other organizations were involved as partners?**
- **Nothing to Report**
- **SPECIAL REPORTING REQUIREMENTS**
 - **COLLABORATIVE AWARDS: N/A**
 -
 - **QUAD CHARTS: Attached**
- **APPENDICES: Nothing to report**

Central pain mechanisms and novel therapeutic strategies in a model of closed head injury



PI: Melanie Elliott, PhD

Org: Thomas Jefferson University

Award Amount: \$1,446,781.80

Study/Product Aim(s)

- **Specific Aim 1:** To identify the pattern of inflammation-induced sensitization of the central trigeminal pain neurons, and if sensitization is detectable by quantitative EEG.
- **Specific Aim 2:** To determine if anti-inflammatory mechanisms inhibit chronic sensitization of central trigeminal pain neurons in vitro.
- **Specific Aim 3:** To assess the in vivo therapeutic efficacy of a novel, non-psychoactive cannabinoid agent in a model of post-traumatic headache.

Approach: (1) Single/repeated mild closed head injury (CHI) will be induced in rats. Markers of inflammation and neuronal activation will be assessed in pain regions. Sensory testing will be compared to pain markers. EEG will be performed during exposure to a variety of sensory stimuli. (2) In vitro brain slices from injured or control groups will be bathed in inflammatory solutions, with or without anti-inflammatories, and the supernatant and tissues analyzed. (3) In vivo drug testing for a novel anti-inflammatory agent will be performed in CHI groups and compared to vehicle-treated groups.

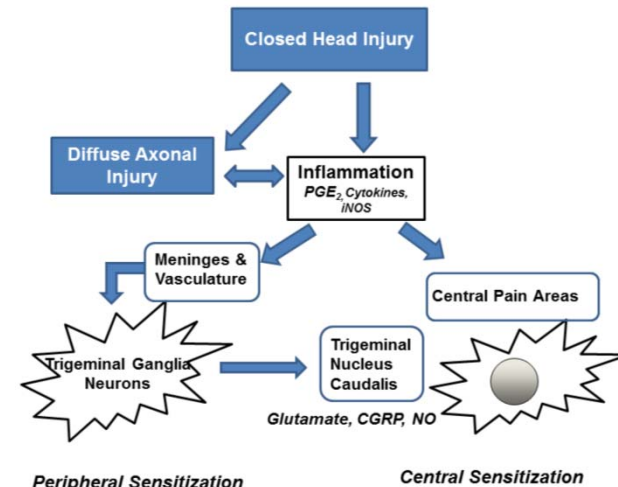


Figure: Proposed mechanisms of post-traumatic sensitization of the trigeminal pain circuit. Prostaglandins (PGE₂), inducible nitric oxide synthase (iNOS), calcitonin gene-related peptide (CGRP),

Timeline and Cost

Activities	CY	14	15	16	17
Aim 1					
Aim 2					
Aim 3					
Estimated Budget (\$K)		\$552K	\$443K	\$451K	

Updated: (October 30, 2015)

Goals/Milestones

CY14-15 Goal – Pain mechanisms and diagnosis

- ☐ Determine altered inflammatory and neuronal markers in the pain pathway in a model of CHI.
- ☐ Develop quantitative EEG to assess sensory changes in a model of CHI.

CY15-16 Goals –Anti-inflammatory strategies in vitro

- ☐ Determine the role of inflammatory stimuli in chronic neuronal sensitization implicated in pain.
- ☐ Determine the best anti-inflammatory strategy to minimize neuronal sensitization implicated in pain.

CY16-17 Goal – In vivo novel anti-inflammatory treatment efficacy

- ☐ Test novel and classical anti-inflammatories in an in vivo model of closed head injury